PATENT APPLICATION

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February 3, 2003
Date

Lara Russell
Lara Russell

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Sprecher, Cindy A., Novak, Julia E., West, James W., Presnell, Scott R., Holly, Richard D., and Nelson, Andrew J.
Application No.	:	09/825,561
Filed	:	April 3, 2001
For	:	SOLUBLE ZALPHA11 CYTOKINE RECEPTORS
Examiner	:	Ruixiang Li
Art Unit	:	1646
Docket No.	:	00-22

Declaration Under 37 CFR § 1.131

Sir:

We, Julia E. Novak, Cindy A. Sprecher, James W. West, Scott R. Presnell, Richard D. Holly, Andrew J. and Nelson, and Julia E. Novak, hereby declare as follows:

1. We are the named inventors on the above-identified application and have reviewed and understand the specification and claims of the above-identified application.

2. All of the work described herein and illustrated by the attached Exhibits was performed in the United States under our direction.

3. I have read and understood the reference cited by the Office, Presnell et al., WO 00/17235, published March 30, 2000.

4. Exhibit 1 comprises copies of pages and figures describing data from a draft manuscript prepared by one of us (Julia E. Novak) submitted in-house for review prior to March 30, 2000. Figure 3 comprised a summary of the data prepared by one or more of us (James E. West and Andrew J. Nelson) whose work is described in detail below and provided in Exhibits 2 and 3. This draft manuscript establishes that the invention of subject matter of the above-identified application, i.e., heterodimeric and multimeric zalpha11-containing receptors, occurred prior to March 30, 2000, the publication date of the cited reference.

5. Exhibit 1 (including figure legend "Figure 3." and accompanying Figures 3A and 3B) includes experimental data that establishes the invention of subject matter of the above-identified application, i.e., heterodimeric zalpha11-containing receptors, prior to March 30, 2000. Please note that at the time this manuscript was written we initially used "IL-19R," as opposed to the published "IL-21R" as nomenclature for the zalpha11 receptor polypeptide. Similarly, the nomenclature "IL-19" was used for the ligand (zalpha11Ligand), as opposed to the published "IL-21." Assays were designed and carried out to test whether zalpha11Ligand (IL-21) might in addition to the IL-21R (zalpha11) use the IL2 receptor common gamma chain (γc), a.k.a. IL-2Rγ, as part of its receptor complex, since the cytokines most closely related to zalpha11Ligand (IL-21) utilize this subunit.

(a) ORIGEN Assay Data (see enclosed Figure 3A, and legend). The ORIGEN electrochemiluminescence (ECL) technology (Igen, Inc.) provided a method for measuring dimerization of two differentially labeled proteins in the presence of an unlabeled third protein. A ruthenium metal chelate (Ru) label on one protein will emit luminescence when brought near an electric field through dimerization with a biotinylated protein which is held in place via streptavidin-coated magnetic beads. Using this assay, we assessed the dimerization of the soluble zalpha11 receptor (IL-21R; denoted as IL-19R in Exhibit 1) with various soluble class I cytokine receptor subunits in the presence of the zalpha11Ligand, IL-21 (denoted as IL-19 in Exhibit 1). Homodimerization of the zalpha11 receptor (denoted as IL-19R in Exhibit 1) did not occur in the presence of the zalpha11Ligand (IL-21; denoted as IL-19 in Exhibit 1) using this assay. However,

in this assay, ligand-mediated dimerization of Ru-IL21R (denoted as Ru-sIL19R in Exhibit 1) with bio-IL21R (denoted as bio-sIL19R in Exhibit) or of Ru-IL2Rγ (Ru-sγc) with bio-IL21R (denoted as bio-sIL19R in Exhibit) was measured. The results of this assay showed that zalpha11 receptor (IL-21R; denoted as IL-19R in Exhibit 1) and IL-2Rγ dimerized in the presence of IL21, but not IL2, IL4, or IL15 (Figure 3A); hence the dimerization of zalpha11 receptor (IL-21R; denoted as IL-19R in Exhibit 1) and IL-2Rγ was specific to IL21.

(b) Costimulation Data (see enclosed Figure 3B, and legend). In addition to the ORIGEN assay described above, experiments were conducted in order to determine whether the dimerization of zalpha11 receptor and IL-2Rγ was necessary for signal transduction, neutralizing monoclonal antibodies to IL-2Rγ (anti-γc antibodies) were used in proliferation assays with normal murine splenic B cells. The addition of the anti-γc antibodies TUG/m2 and 3E12 partially blocked proliferation induced by IL21 (denoted as IL-19 in Exhibit 1) and anti-CD40 (Figure 3B), suggesting that IL-2Rγ plays a role in IL21 signal transduction in B cells. This data further supported that the zalpha11 receptor forms a functional complex with the IL2 receptor common chain (γc), a.k.a. IL-2Rγ.

(c) Multiple subunits. At least one of us (Julia E. Novak) recognized prior to March 30, 2000 that the IL2 receptor had been studied in detail and is composed of an α-β-γc heterotrimer. The β and γc subunits are both essential for signal transduction and are members of the hematopoietin receptor superfamily, whereas the α subunit appears to primarily be involved in high-affinity binding conversion and is structurally distinct from the hematopoietin receptor family. The γc subunit has been shown to participate in forming the receptors for IL4, IL7, IL9, and IL15, in addition to IL2 (for review, see Sagamura, K. et al., Ann. Rev. Immunol. 14: 179-205 (1996); copy enclosed)). Based on what was known about other Class I cytokine receptors, we recognized prior to March 30, 2000 that not only could zalpha11 receptor form a heterodimeric complex with γc as we had demonstrated, but that it would not be unreasonable to form a trimeric or

multimeric complex, for example, comprising other Class I cytokine receptor subunits, for example, in addition to the IL-2Rγ receptor.

6. The experiments summarized in Exhibit 1, performed prior to March 30, 2000, describe and provided experimental evidence for a functional zalpha11 receptor complex that contains the IL2 receptor common chain (γc), a.k.a. IL-2Rγ. Our data suggested that IL21 acts through a receptor complex that includes zalpha11 receptor and the γc subunit of IL2R, even though the cytoplasmic domain of zalpha11 receptor was capable of transducing signal in a homodimeric configuration (e.g., see Novak et al., US Patent No. 6, 307, 204; cited by Office). This finding was similar to the known receptor, IL4Rα, which is also capable of signaling as a homodimer (Kammer, W. et al.., J. Biol. Chem. 271: 23634-23637 (1996); copy enclosed), although the natural functional IL4 receptor complex is a IL4Rα/γc heterodimer.

7. Exhibit 2 comprises copies of notebook pages 42 and 103 from ZymoGenetics Notebook #6917, and pages 130, 138-142 from ZymoGenetics Notebook #6637, describing data prepared by one of us (James W. West) prior to March 30, 2000. The following experiments were designed to ask whether IL2Rγ was a component of the zalpha11 receptor complex, which binds zalpha11Ligand (IL21). We used an ORIGEN dimerization assay as described in paragraph 5(a) above.

(a) Pages 42 and 103 from ZymoGenetics Notebook #6917. These pages show an ORIGEN assay where IL21 (denoted as "IL-19", "zalpha11Lig" or "α11lig" in the notebook) promoted the dimerization of the zalpha11 receptor (denoted in shorthand as "zα11" in the notebook) and IL2Rγ. In contrast IL2, IL4 and IL15 did not demonstrating specificity. These experiments were summarized in the data graph as shown at the bottom of page 103 (this was the graph used for figure 3A in Exhibit 1). The results of this assay showed that zalpha11 receptor and IL2Rγ dimerized in the presence of IL21, but not IL2, IL4, or IL15 (Figure 3A); hence the dimerization of zalpha11 and IL2Rγ was specific to IL21.

(b) Pages 130, 138-142 from ZymoGenetics Notebook #6637. Previous experiments to those described above (Paragraph 7(a)) using the same ORIGEN dimerization assay described herein showed that IL-21 (denoted as "zalpha11Lig"

or "α11lig" or "zalpha11 ligand" in the notebook) in conditioned media from clones expressing IL-21 promoted dimerization of zalpha11 and IL-2Rγ. Page 130, and 138-142 shows that 40X concentrated IL-21 containing media prepared by one of us (Cindy A. Sprecher) from clones expressing "zalpha11 ligand" (Page 130) promoted the dimerization of zalpha11 receptor and IL-2Rγ in the ORIGEN dimerization assay. Pages 138-139 show that the receptor dimerization assay results showed heterodimerization of zalpha11 receptor and IL-2Rγ in the presence of zalhpa11Ligand conditioned media. Pages 140-141 showed a repeat of these results showing heterodimerization of zalpha11 and IL-2Rγ in the presence of IL-21 ("zalpha11 lig"). The ORIGEN electrochemiluminescence (ECL) technology (Igen, Inc.) provided a method for measuring dimerization of two differentially labeled proteins in the presence of an unlabeled third protein. A ruthenium metal chelate (Ru) label on one protein will emit luminescence when brought near an electric field through dimerization with a biotinylated protein which is held in place via streptavidin-coated magnetic beads. Using this assay, we assessed the dimerization of zalpha11 receptor with various class I cytokine receptor subunits in the presence of the IL-21 (zalpha11Ligand). Homodimerization of zalpha11 receptor did not occur in the presence of IL-21 (zalpha11Ligand) using this assay, nor did heterodimerization with IL-4R or with IL-4R and IL-2Rγ. However, in this assay, ligand-mediated dimerization of zalpha11 with bio-IL-2Rγ was shown.

8. Exhibit 3 comprises copies of notebook pages 36-41 from ZymoGenetics Notebook #7072 describing data prepared by one of us (Andrew J. Nelson) prior to March 30, 2000. These notebook pages have the raw data and results of experiments used to support the "Costimulation Data" described in Figure 3B, and legend in Exhibit 1. Pages 36 and 37 described the isolation of splenic B-cells, pre-incubation with the anti-γc antibodies (TUG/m2 and 3E12) and co-stimulation of the B-cells with IgM or andt-CD40 antibodies in the presence of either murine zalpha11-Ligand (a.k.a., IL-21 or "IL-19" as described above), mIL2, mIL4 or

mIL15 at concentrations listed. Sixteen hours prior to harvesting, 1 μCi ^{3}H-thymidine (Amersham, Piscataway, NJ) was added to all wells to assess whether the B-cells had proliferated. The raw data from the TopCount Microplate Scintillation Counter (Packard) showing ^{3}H-thymidine-incorporation and hence proliferation of the B-cells in the presence of the various cytokines is shown on page 37-38; and the corresponding summary via graphic representations of each experiment are presented on pages 38-41. Specifically, the results shown on page 38 ("7072.36 anti CD40 w/ titrating amounts of zalpha11Lig w/wo Rx of TUG & 3E12 cells CD19 pos select from frozen PBMC") showed that the addition of the anti-γc antibodies partially blocked proliferation induced by the zalpha11-Ligand (IL-21) and anti-CD40 (also shown in Figure 3B in Exhibit 1), which suggested that the IL2 receptor common chain (γc), a.k.a. IL-2Rγ.played a role in zalpha11-Ligand (IL-21) signal transduction in B cells. This data further supported that the zalpha11 receptor formed a functional complex with the IL2 receptor common chain (γc), a.k.a. IL-2Rγ.

9. The data summarized in Exhibits 1-3 showed the conception of heterodimeric and multimeric zcytor11-comprising receptors, as well as the actual reduction to practice of at least one heterodimeric receptor complex containing an isolated soluble receptor polypeptide comprising a sequence of amino acid residues as shown in SEQ ID NO:6 (zalpha11 soluble receptor), wherein the soluble receptor polypeptide forms a heterodimeric receptor complex; as well as the receptor complex further comprising other Class I cytokine receptor subunits, for example, the IL-2Rγ receptor.

10. On the basis of these Exhibits, which document activities within the United States of America, we conclude that the invention described in claims 31-33, 35, 37, and 48-52, of the above-captioned application was conceived and reduced to practice prior to March 30, 2000.

11. We further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

By _____ Date 1/13/2003
Julia E. Novak

By _____ Date Jan. 23, 2003
~~Cyndi~~ Cindy A Sprecher
Cindy

By _____ Date _____
James W. West

By _____ Date JAN 13, 2003
Scott R. Presnell

By _____ Date 1-13-03
Richard D. Holly

By _____ Date 1/13/03
Andrew J. Nelson